Clustering of Environments of Southern Soft Red Winter Wheat Region for Milling and Baking Quality Attributes

A. Collaku,* S. A. Harrison, P. L. Finney, and D. A. Van Sanford

ABSTRACT

Division of regional nursery test sites into homogenous subregions contributes to more efficient evaluation and better differentiation of cultivars. Data from the Uniform Southern Soft Red Winter Wheat Nursery (USSRWWN) were analyzed to group testing sites into relatively homogenous subregions for milling and baking quality (MBQ) attributes. Environmental effects due to years accounted for over 50% of the total variation for protein content (P) and 42% for alkaline water retention capacity (AWRC). Genotype effect accounted for 63% of the total variation for softness equivalence (SE), and 37% for flour yield (FLY). A significant genotype × location (G×L) interaction occurred for FLY and P. However, the G×L variance component accounted for a small proportion of the total phenotypic variance, suggesting that clustering would be more beneficial for resource efficiency than for increasing differentiation of genotypes. A hierarchical cluster analysis was used to group locations on the basis of G×L interaction effects for FLY, P, AWRC, and SE. Cluster analysis divided the USSRWWN into two main subregions within which the G×L interaction was reduced by over 90% for FLY and by 60% for P. Although this classification is not entirely consistent with the geographic distribution of locations, clusters do follow general geographic-climatic-disease regions. Our results suggest that the USSRWWN can be divided into subregions to reduce the resources expended on evaluation of MBQ attributes. This classification of locations could be useful in breeding for specific adaptability within subregions.

THE USSRWWN includes environments with diverse moisture supply, temperature, soil type, and biotic stresses. Under such conditions, genotype × location (G×L) interaction is expected to be large and may result in failure to differentiate performance of genotypes across environments. Division of regional trials into subregions with reduced G×L interactions may allow better genotype differentiation and reduce costs. This is particularly important for resource intensive evaluation of traits such as MBQ.

Horner and Frey (1957) divided oat test areas into subareas within which the G×L interaction component of variance was substantially reduced. Since genotype responses are multivariate rather than univariate (Lin et al., 1986), multivariate techniques are generally more effective in explaining G×E interactions (Zobel et al., 1988; Nachit et al., 1992). Among multivariate techniques, cluster analysis based on differences in response of genotypes across environments is the most widely used. Abou-El-Fittouh et al. (1969) used this technique to classify cotton test sites. A number of studies have been conducted in wheat (*Triticum aestivum* L.) to classify locations using cluster analysis (Campbell and Lafever, 1980; Ghadery et al., 1980; Fox and Rosielle, 1982). Yau et al. (1991) used a hierarchical agglomerative and polythetic clustering technique to analyze ICARDA/CIMMYT Regional Bread Wheat Yield Trial data. Van Oosterom et al. (1993) used cluster analysis to study relationships among barley (*Hordeum vulgare* L.) environments in the Mediterranean Region. Peterson and Pfeiffer (1989) and Peterson (1992) used principal factor analysis to describe wheat location relationships and determine specific production zones for hard red winter wheat cultivars. Hanson (1994) developed distance statistics based on the concept of genotypic stability to interpret regional soybean tests.

MBQ traits of wheat are genetically influenced and have been bred into the widely used cultivars accepted as standards (Finney et al., 1987). Environmental conditions also have a significant influence on MBQ traits of wheat (Baenziger et al., 1985; Finney et al., 1987; Bruckner and Finney, 1992; Peterson et al., 1992).

The objectives of this study were to: (i) evaluate magnitude and nature of genotype, location, and G×L interaction effects for MBQ in the USSRWWN; (ii) classify locations of the USSRWWN into clusters to reduce G×L interaction for MBQ attributes; and (iii) develop subregions that allow more efficient evaluation and differentiation of wheat genotypes for MBQ traits.

MATERIALS AND METHODS

Data from 1992, 1993, and 1994 harvest year of the USSRWWN were analyzed as part of a cooperative study for MBQ evaluation of soft red winter wheat breeding lines and cultivars. Trials were conducted at 16 locations: Overton, TX; Baton Rouge, LA; Keiser, AR; Cleveland, MS; Belle Mina, AL; Griffin, GA; Quincy, FL; Clemson, Florence and St. Mathew, SC; Clayton, NC; Warsaw, VA; Quantico, MD; Landisville, PA; Princeton, KY; and Knoxville, TN. Ten entries (genotypes) from a total of 23 to 44 entries of the 1992 USSRWWN were considered as a representative sample and were also included in the 1993, and 1994 USSRWWN to be used for this study. The entries chosen had different genetic background and belonged to different locations of origin within the southern soft red winter wheat region. However, because of a small sample size of only 10 entries, estimates of genetic variances must be interpreted with caution. Experiments in each location were conducted according to a random-

A. Collaku, Pennington BRC, Louisiana State Univ., 6400 Perkins Rd., Baton Rouge, LA 70808; S.A. Harrison, Dep. of Agronomy, Louisiana Agric. Exp. Stn., Louisiana State Univ., Agricultural Center, Baton Rouge, LA 70803; P.L. Finney, USDA-ARS, Soft Wheat Quality Lab., Ohio Agric. Res. and Dev. Center, Wooster, OH 44691. D.A. Van Sanford, Dep. of Agronomy, University of Kentucky, Lexington, KY 40506. Approved for publication by the Director of the Louisiana Agric. Exp. Stn. as manuscript no. 00-09-0689. Received 4 Dec. 2000. *Corresponding author (collaka@pbrc.edu).

ized complete block design with two to four replications and varied plot size.

Grain samples were provided by nursery cooperators each year and were analyzed for milling and baking quality attributes at USDA-ARS Soft Wheat Quality Laboratory, Wooster, OH. Samples were lightly cleaned to remove shriveled, broken, and/or disease-damaged kernels before being analyzed. Milling quality was based on flour yield (FLY) from a 25-g micro milling, whereas baking quality was assessed from flour protein concentration (P), alkaline water retention capacity (AWRC), and softness equivalence (SE), (Yamazaki and Donelson, 1972; Finney, 1992).

A combined analysis of variance across locations and years was conducted for each of four quality traits to test the significance of G×L interaction and evaluate the relative importance of different factors on MBQ traits. Locations were considered as representative of the USSRWWN region, whereas entries were considered a representative sample of the entries being tested from 1992 through 1994. Therefore, analyses of variance were conducted assuming a random model with unbalanced data, using Proc GLM of SAS (SAS Institute, 1996). In testing genotypes and G×L interaction, approximate F test were computed, as:

$$F(Gen) = MS\ (Gen)/0.98MS(G×L)$$
$$+ 0.01MS[Gen × Yr(Loc)]$$
$$+ 0.01MS(Error)$$
$$F(G×L) = MS(G×L)/0.99MS[Gen × Yr(Loc)]$$
$$+ 0.01MS(Error)$$

G×L interaction effects were calculated as:

$$\overline{X}_{ij} - \overline{X}_{i.} - \overline{X}_{.j} + \overline{X}_{..}$$

where:

$\overline{X}_{ij}$ = entry mean of the jth genotype
in the ith location
$\overline{X}_{i.}$ = mean of the ith location
$\overline{X}_{.j}$ = mean of the jth genotype
$\overline{X}_{..}$ = overall mean

Cluster analysis was used to group locations according to similarities of G×L interaction effects. A series of MBQ traits was used simultaneously to identify the most useful parameters in the division of subregions. A hierarchical cluster analysis using Ward's method algorithm (Ward, 1963), with the sum of squares between the two clusters added up across all the variables as the distance measure and prediction ratio (PR)

as clustering strategy was employed. Prediction ratio is defined as follows:

$$PR = (1 - R^2)^{1/2}$$

where R^2 = squared multiple correlation, which is the sum of squares between all the clusters divided by the corrected total sum of squares.

Cluster analyses were performed using SAS Proc CLUSTER and TREE (SAS Institute, 1996). The hierarchical clustering was truncated at the stage corresponding to the initial sharp decline of R^2. For each group of locations resulting after truncation, a combined analysis of variance across locations and years was performed for each trait separately. Efficiency of clustering for different character combinations was evaluated by the percentage reduction of G×L variance component within clusters as compared to the G×L variance component of all the locations considered together.

RESULTS

Genotypic effect was significant for all the four MBQ traits (Table 1). Genotypes accounted for 37% of total variability for FLY (Table 1) and the range of the means among the 10 genotypes was from 69.6 to 72.6% (Table 2). For P, genotypes accounted for 20% of the total variability with a range of means from 8.6 to 10.6%, which is typical of soft red winter wheat. Across the 16 locations SE exhibited a wide range of variability, of 39.7 to 61.1 for Pioneer 2684 and 46.6 to 63.9 for Florida 302. Genotypes accounted for 63% of the total variability of SE. Environmental components, particularly years, were highly significant ($P < 0.01$) and had a strong effect on almost all MBQ traits. Years accounted for 54% of the total variability of P and 42% of total variability for AWRC (Table 1). The G×L interaction was significant for FLY, P ($P < 0.01$), and AWRC ($P < 0.05$), but its magnitude was small relative to other components because of a large year effect. The G×L variance component accounted for 16% of phenotypic variance for FLY and 6% for P. Therefore, clustering of locations based on G×L interaction would be more for achieving resource efficiency than for improving differentiation of genotypes.

Locations of USSRWWN were classified into four groups when clustering for the G×L interaction effects of all four MBQ traits (Fig. 1a) and into three groups when clustering considered FLY, P, and AWRC (Fig.

Table 1. Analyses of variance of flour yield (FLY), protein concentration (P), alkaline water retention capacity (AWRC), and softness equivalence (SE) for 10 wheat entries across 16 locations of the Uniform Southern Soft Red Winter Wheat Nursery in 1992–1994.

Source	df	FLY			P			AWRC			SE		
		MS	$\hat{\sigma}^2$	%†	MS	$\hat{\sigma}^2$	%†	MS	$\hat{\sigma}^2$	%†	MS	$\hat{\sigma}^2$	%†
Reps within Loc × Yr	40				0.61			2.06			2.37		
Location (Loc)	15	26.01**	0.33	14	27.93**	0.18	10	25.95	–	–	262.04**	2.07	5
Years (Yr) within Loc	27	10.05**	0.53	23	18.68**	0.99	54	40.81**	2.13	42	159.18**	8.3	19
Genotypes (Gen)	9	60.19**	0.84	37(95)	27.09**	0.35	20	104.83**	1.37	27	2085.74**	27.59	63
Gen × Loc	135	1.16**	0.14	6(16)§	0.50**	0.04	2(6)§	1.91*	0.05	1(3)§	9.34	0.09	0.2(0.3)§
Gen × Yr within Loc	229	0.79	0.09	4	0.32**	0.09	5	1.67*	0.17	3	8.96	3.2	7
Residual	334	0.38		16	0.15		7	1.34		26	2.85		5.8
Phenotypic variance			0.89			0.65			1.44			28.68	

* Indicates significance at $P = 0.05$.
** Indicates significance at $P = 0.01$.
† Percentage of total variability.
§ Number in parenthesis is percentage of Genotype × Location variance component as compared to phenotypic variance.

Table 2. Milling and baking quality of 10 soft red winter wheat genotypes across 16 locations of the Uniform Southern Soft Red Winter Wheat Nursery in 1992–1994.

| Genotype	FLY† (%)		P‡ (%)		AWRC§		SE¶	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Florida 302	72.6	69.0–75.4	9.6	7.2–12.1	54.9	51.7–58.8	55.2	46.6–63.9
Saluda	71.3	67.5–73.5	9.5	6.9–12.1	56.6	52.1–60.7	56.5	46.8–67.0
VA 88-52-69	70.6	67.4–72.6	9.5	7.2–12.5	57.3	53.8–62.6	53.3	41.6–60.5
GA8012-26-12	70.2	66.9–72.7	9.7	6.8–12.7	55.4	50.6–60.1	53.9	45.0–61.2
Pioneer 2684	70.5	66.0–72.7	8.9	6.7–12.5	56.9	52.5–60.6	51.8	39.7–61.1
Coker 9835	71.3	68.3–73.4	8.6	6.6–12.2	56.3	53.2–60.7	60.1	51.6–68.7
MD 80004-62	71.0	67.7–73.6	9.4	7.0–13.0	57.3	53.7–60.8	40.8	35.3–55.9
SC 870196	70.8	65.7–73.2	10.5	7.9–13.8	57.0	52.4–62.1	50.3	41.7–57.2
TX 885-121-2	69.6	65.5–72.7	10.6	7.7–13.9	58.9	55.3–62.0	48.5	40.4–58.1
AL881060	72.3	69.2–74.4	9.9	7.6–13.7	55.1	50.8–58.6	52.0	44.2–60.3

† Flour Yield.
‡ Protein content.
§ Alkaline Water Retention Capacity.
¶ Softness Equivalence.

1b). Clustering of locations reduced G×L interaction within each cluster. A nonsignificant within-clusters G×L variance resulted for FLY, P, and AWRC. The magnitude of G×L interaction within clusters was substantially reduced compared to G×L interaction for all the locations considered together (Table 3).
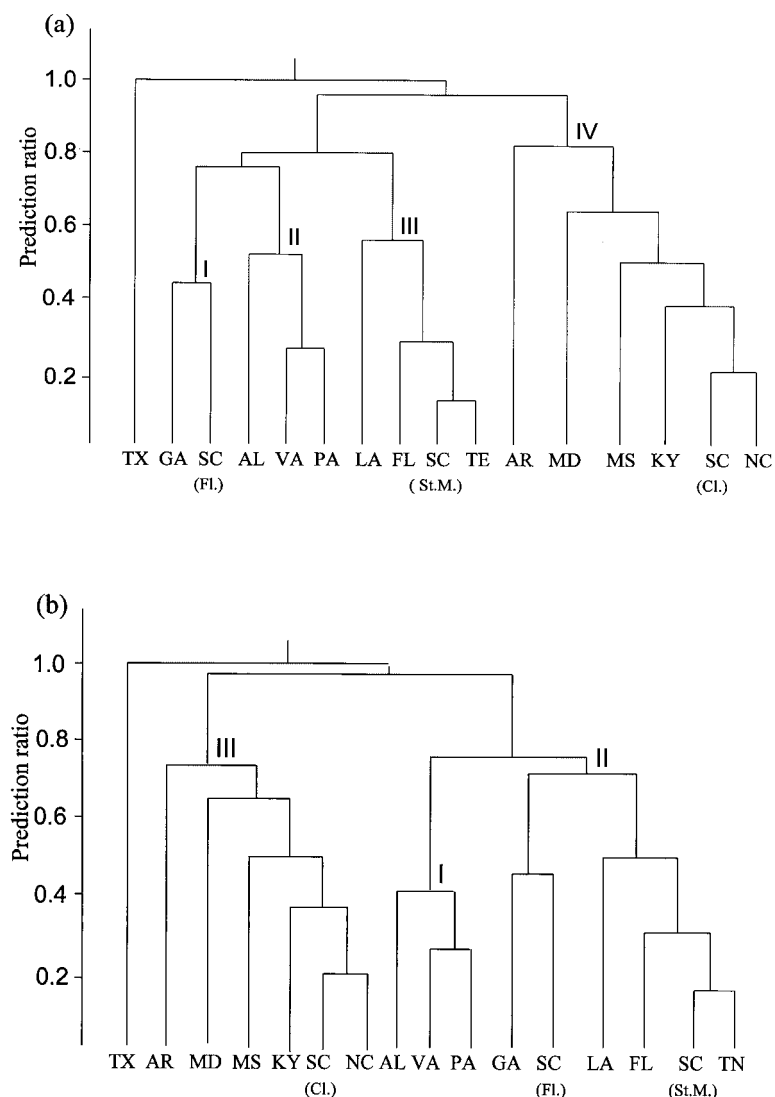
Fig. 1. Dendrograms from clustering 16 locations of the Uniform Southern Soft Red Winter Wheat Nursery in 1992–1994 for (a) flour yield, protein content, alkaline water retention capacity, and softness equivalence; (b) flour yield, protein content, and alkaline water retention capacity.

Table 3. Genotype × Location variance components for flour yield (FLY), protein concentration (P), alkaline water retention capacity (AWRC), and softness equivalence (SE) within and across clusters.

| Traits | Genotype × location variance component | | | | | | |
	All locations	Cluster I	Cluster II	Cluster III	Cluster IV	Pooled over clusters	Reduction (%)†
	Clusters based on FLY, P, AWRC, and SE						
FLY	0.140**	0.001	0.027	0.002	0.008	0.0087	93
P	0.040**	0.013	0.004	0.001	0.004	0.0044	89
AWRC	0.050*	0.660	0.050	0.000	0.001	0.0984	−97
SE	0.090	0.000	0.027	0.000	0.004	0.0070	91
	Clusters based on FLY, P, and AWRC						
FLY	0.140**	0.027	0	0.008		0.0080	94
P	0.040**	0.004	0.034	0.004		0.0160	60
AWRC	0.050*	0.050	0.022	0.001		0.0192	62
SE	0.090	0.027	0.257	0.004		0.1098	−22

* Indicates significance at $P = 0.05$.
** Indicates significance at $P = 0.01$.
† Pooled G×L variance components within clusters in percentage of G×L variance component across all the locations.

Three groups of locations (clusters) resulted from clustering based on FLY, P, and AWRC: (i) Belle Mina (AL), Warsaw (VA), Landisville (PA); (ii) Griffin (GA), Florence (SC), Baton Rouge (LA), Quincy (FL), St. Matthew (SC), and Knoxville (TN); and (iii) Keiser (AR), Cleveland (MS), Princeton, (KY), Clemson (SC), Clayton (NC), and Quantico (MD). The location of Clusters I and III were consistent with the clustering results based on FLY, P, AWRC, and SE (Fig. 1a), which corresponded to Clusters II and IV. Locations of Cluster II when only FLY, P, and AWRC were considered (Fig. 1b) were classified into two separate clusters (Cluster I and III) when all the four MBQ traits were considered (Fig. 1a).

In all cases, Overton (TX) remained separate from all other locations, in part caused by dissimilar geographical and soil conditions as compared to the other locations of USSRWWN.

In the case of three traits (FLY, P, and AWRC), clusters were formed earlier than in the case when four traits (FLY, P, AWRC, and SE) were considered. When clustering for three traits, the amalgamation distance (PR) was 0.15 lower for Cluster I and 0.18 lower for Cluster III as compared to the respective PR of Clusters II and IV in the case of four traits (Fig.1a, b).

Sixty-three percent of the variation of SE was attributed to genotypic effects, compared to G×L interaction which was 0.3% of the phenotypic variance (Table 1), suggesting that SE would be of little value in G×L clustering.

Results of the relative reduction of G×L interaction within clusters for FLY were similar in both clustering procedures. Within-cluster G×L variance for P was reduced by an average of 89% when clustering for four traits as compared with 60% when clustering for three traits (Table 3). Although G×L interactions of AWRC were reduced in most of the clusters, its average reduction was negative when clustering with four traits due to a very large increase in Cluster I.

When clustering was based on three traits, G×L interactions were reduced more for FLY (93%, Table 3) than for P and AWRC (60 and 62%, respectively, Table

3). The increase of 22% in SE G×L interaction variance was nonsignificant.

The distribution of locations within Clusters II and III when clustering for FLY, P, and AWRC, corresponded to their geographic and climacteric characteristics (Fig. 1b and Fig. 2). The mean latitude for Cluster II was 32.9°N (Table 4). Most of the locations in this cluster belong to the southern and southeastern coastal part of the USSRWWN region, with a latitude from 30.3 to 34.1°N. However, locations such as Knoxville (TE) at 36.0°N were included in this cluster, demonstrating that latitude was not the only factor influencing the division of zones for southern soft red winter wheat. Cluster III included locations ranging in latitude from 33.8°N to 37.1°N (Table 4), with the exception of Quantico (MD) with a latitude of 39.5°N. The mean latitude of locations in Cluster III was 36.1°N (Fig. 2). Cluster I included two locations from the northern part of the region: Landisville (PA) and Warsaw (VA), with latitudes ranging from 38 to 40.1°N along with Belle Mina (AL) with a latitude of 34.8°N (Table 4). Because of only three locations, this group should not be recognized
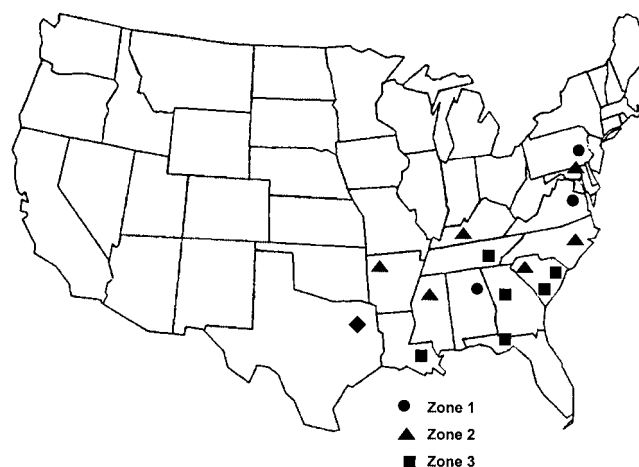
Fig. 2. Zones for soft red winter wheat in South-Eastern Region from clustering for flour yield, protein content, and alkaline water retention capacity.

Table 4. Latitude of 16 locations of the Uniform Southern Soft Red Winter Wheat Nursery in clusters based on flour yield, protein concentration, and alkaline water retention capacity.

Locations within clusters	Latitude °N
Cluster I	
Belle Mina (AL)	34.8
Warsaw (VA)	38.0
Landisville (PA)	40.1
Mean	37.6
Cluster II	
Griffin (GA)	33.2
Florence (SC)	34.1
Baton Rouge (LA)	30.3
Quincy (FL)	30.4
St. Matthew (SC)	33.7
Knoxville (TE)	36.0
Mean	32.9
Cluster III	
Kaiser (AR)	35.7
Quantico (MD)	39.5
Cleveland (MS)	33.8
Princeton (KY)	37.1
Clemson (SC)	34.8
Clayton (NC)	35.6
Mean	36.1
Overton (TX)	32.5

as a differentiate zone. More representative locations are needed to decide future divisions within this zone.

DISCUSSION

If test sites are not representative of the target environment, a large G×L interaction may result and hinder the progress of the breeding program. The significant G×L interaction for FLY, P, and AWRC implied that the USSRWWN region can be divided into more homogeneous subregions for the purpose of achieving resource efficiency for MBQ traits. This result was expected because the USSRWWN region includes locations with diverse moisture supply, temperature, soil type, and biotic stresses. Similar results have been reported in studies with yield data involving large and heterogenous areas in wheat (Campbell and Lafever, 1980; Ghadery et al., 1980; Fox and Rosielle, 1982; Yau et al., 1991).

In contrast with other studies where cluster analysis was used to classify locations on the basis of a single trait (Campbell and Lafever, 1980; Ghadery et al., 1980; Fox and Rosielle, 1982; Collaku, 1991; Van Oosterom et al., 1993), this study is based on different combinations of MBQ traits. When classification of locations involves G×L interactions of traits such as milling and baking quality, it is important to consider all traits together. The analysis of milling and baking quality attributes are costly and more effective selection of test sites with representative locations from each subregion should reduce the necessary cost of evaluation.

Cluster analysis divided the USSRWWN region into subregions with similar locations (Fig. 2). This classification is not consistent with the geographic distribution of locations, although there is a tendency for clusters to follow general geographic-climatic-disease regions. Environmental variation due to weather conditions is often considered as a major factor influencing quality

traits in wheat, and this seems to be true in this study. The environmental component of years had the largest effect on the variation of P, and AWRC. Its effect was important on FLY (23%) and SE (19%), as well. Among the three clusters, two of them corresponding to Cluster II and Cluster III in Fig. 1b, were more distinct. Cluster II included mainly test sites of locations across the coastal south and southeastern region, that is characterized by mild temperatures and similar biotic stresses. Cluster III grouped together test sites from the central part of the southern region with more severe temperatures and other related climatic and biotic conditions different from those of Cluster II. The most relevant diseases of the region, such as leaf rust (*Puccinia recondita* Roberge ex Desmaz), stem rust (*Puccinia graminis* Pers.:Pers.), and septoria [*Mycosphaerella graminicola* (Fuckel) Schöter], which are dependant on the temperature and moisture supply, may have influenced the division of these two subregions. The third group of locations corresponding to Cluster I (Fig. 1b), cannot be considered as a complete subregion because it fused only a few very different locations, two from the northern part of the region along with one from the southern part.

The results of this study support the idea that the USSRWWN region should be divided into more similar subregions. If wide adaptability is the main breeding objective, representative locations from the southern and central zones (Cluster II and III) along with other locations of the USSRWWN should be chosen. This could help in a better distribution of resources across locations with the needed diversity. On the other hand, if specific adaptability were the primary goal, then resources and efforts can be concentrated within the subregion of interest. More intensive efforts (more locations in less years) could be concentrated within a specific subregion to evaluate and release new cultivars with improved MBQ attributes.

Another implication should be in the testing procedure. Increased cost efficiency can be obtained by selecting locations from each subregion to test for MBQ within wheat genotypes. However, reduction in number of locations has the risk of losing information. Therefore, in reducing the number of locations, one should carefully consider only those similar locations that are close in the clustering stages.

Deviations from the proximity of test sites were found in each cluster. Besides the specific features of locations, a major factor influencing these deviations was the use of G×L interaction as a measure of similarity, instead of environmental indexes. Our results confirm those of Baenziger et al. (1985) and Peterson et al. (1992) where they reported significant variation in quality traits attributed to G×L interactions. A greater emphasis on G×L interaction of quality traits would be beneficial for a better differentiation of wheat genotypes, as well as in the classification of environments useful in selection of test sites.

Classification analysis of related traits such as MBQ attributes should consider the set of single traits simultaneously in a multivariate approach. In this study, a hier-

archical cluster analysis based on G×L interaction of four quality traits proved an effective means for subdividing a variable region such as USSRWWN into more uniform subregions.

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